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Pyruvate kinase activity in the placentas of women living in polluted and unpolluted environments

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A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
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Summary

Background: It was shown earlier that in women living in a polluted environment, the proportion of hypotrophic newborns was greater than in the general population. The placentas of these women show significant histological changes. The authors decided to check whether the activity of pyruvate kinase (PK), a key enzyme in the third stage of glycolysis, changes similarly.

Material/Methods: The study was conducted on placentas collected from women who had lived in a polluted environment (Groups I and II) and those who had inhabited an unpolluted area (Group III) while pregnant. Total PK activity and the specific activities of its isoenzymes were measured according to the Bücher and Pfleiderer method. The isolation of PK isoenzymes was carried out following the previously described procedure.

Results: Total PK activity was lower in the placentas of women of Groups I and II than in those of Group III. The presence of two PK isoenzymes, M_2 and L, was demonstrated. The M_2 and L isoenzymes from the placentas of Groups I and II had a lower specific activity than those of Group III. Only one form of the M_2 isoenzyme and the L dephospho-isoenzyme in the placentas from Groups I and II was observed, while in placentas of Group III women both forms of the M_2 isoenzyme and the L phospho-isoenzyme were noted.

Conclusions: It is possible that the presence of the two PK isoenzymes in the placenta ensures the production of a sufficient amount of pyruvate.

key words: **pyruvate kinase (PK) • ATP • L-cysteine • placenta • air pollution**

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BACKGROUND

As is commonly known, the human placenta is a fast-growing organ that guarantees a regular supply of oxygen and nutritive substances from the mother's circulation to the fetus and also removes metabolic waste products from the fetal circulation. It has been shown that in women living in the polluted environment of Chorzów, where concentrations of various toxic substances in the air are higher than normal, the proportion of hypotrophic newborns with birth weights below 2500 g was greater than in the general number of newborn infants [1,2]. It has also been demonstrated that the placentas of pregnant women living in a polluted environment show histological changes compared with placentas obtained from women living in unpolluted areas. An increased number of villi and a concurrent reduction in their dimensions were reported [3–5]. Moreover, lactate dehydrogenase activity was lower than normal in the placentas of women inhabiting polluted areas [6,7]. This observation is significant, as the placenta is the organ where a major part of glucose is metabolized to lactic acidic, which subsequently penetrates the body of the fetus, where it constitutes an important energy substrate. We might thus infer that such conditions facilitate disturbances in the third stage of glycolysis, including a change in pyruvate kinase (PK) activity [8]. Pyruvate kinase is a key enzyme in this stage of glycolysis, which catalyzes the formation of pyruvate which is, in turn, a substrate for lactate dehydrogenase.

Pyruvate kinase (E.C.2.7.1.40) is found in the cytoplasm and, at times, it may be loosely connected to the endoplasmic reticulum. The enzyme catalyzes the last, irreversible reaction of the third stage of glycolysis, in which pyruvate and ATP are formed from 2-phosphoenolopyruvate and ADP. Four PK isoenzymes have been shown to occur in human tissues and have been designated as M_1, M_2, L, and R [9]. The PK isoenzymes are tetramers. Dimers may also occur in the case of the M_2 isoenzyme. Tetramers are characterized, however, by a higher affinity to the basic substrate, i.e. 2-phosphoenolopyruvate [10,11]. In particular tissues either one or two of the above isoenzymes may be found [9–12]. With the exception of M_1, the isoenzymes are allosteric proteins and hence their activity may be regulated by various positive or negative low-molecular-weight effectors.

Based on the above data, the authors undertook to compare the total placental PK activity in women who had lived in a polluted environment while pregnant to that in the placentas of women who had inhabited relatively pollution-free areas during pregnancy. The investigations were carried out on the placentas obtained immediately after delivery from women living in Chorzów and Kraków, and in the Bieszczady Mountains. These studies were conducted in southern Poland, in the vicinity of the very heavily industrialized Silesian region, and in Kraków, a city situated near Nowa Huta and Skawina, where the air is contaminated with sulfur (IV) oxide, carbon monoxide, lead (+2) and fluoride compounds, and dust. According to the values accepted as normal in Poland, the concentrations of these toxic substances in the air exceed the allowed limits (Table 1).

Spellman and Fotrell had demonstrated previously that the placentas of healthy women contained only one PK isoenzyme, namely M_2 [12]. This isoenzyme is found in numerous tissues, including those characterized by rapid growth [9,12–17]. The authors decided to check whether the activity of the isoenzyme M_2 found in the placentas of women who had lived in polluted areas while pregnant was comparable to its activity in the placentas of women inhabiting pollution-free areas. To date, no such investigations have been carried out; to the best of the authors' knowledge, no attempt has been made to check whether placentas contain yet another PK isoenzyme besides M_2.

Table 1. The annual values of some air pollutants in the areas inhabited by the women belonging to the studied groups [43].

Substance	Acceptable value	Chorzów (Group I)	Kraków (Group II)	The Bieszczady Mountains (Group III)
SO_2 [µg/m^3]	32	44.6	43.0	36.1
CO [mg/m^3]	0.12	3.1	2.0	0.14
NO_2 [µg/m^3]	50	48.2	39.0	16.8
Dust [µg/m^3]	50	94.0	55.5	24.1
Pb^{2+} [µg/m^3]	0.2	0.2	0.3	–
F^- [µg/m^3]	1.6	1.2	1.1	–

MATERIALS AND METHODS

Subjects

The study was carried out on placentas collected from 23 women immediately after delivery at the turn of 1995. These women lived in either polluted and pollution-free areas. In the course of preliminary clinical investigations, the 23 healthy women were selected from a large group. All the women were healthy and tested normal in clinical follow-ups while pregnant. In each woman, the pregnancy was normal and terminated with at-term delivery. In addition, all the pregnant women included into the study were free of alcohol and tobacco addiction. The subjects were divided into three groups. Women who had lived in a polluted environment during pregnancy were assigned to Groups I (Chorzów – 6 placentas) and II (Kraków – 9 placentas). Women living in the non-polluted area were allocated to Group III (the Bieszczady Mountains – 8 placentas) and were regarded as a reference group. In the Bieszczady Mountains, the concentration of air-polluting substances did not exceed the normal values (Table 1).

Materials and Methods

Immediately after delivery and placenta removal, a few samples comprising the whole thickness of the organ were taken from the intermediate zone of each placenta. Immediately afterwards, the samples were frozen on dry ice and stored at –70°C. For estimation of total pyruvate kinase (PK) activity, samples taken from each placenta were homogenized in a Potter-Elvehjem glass homogenizer, in a buffer consisting of 20 mM Tris-HCl, pH 7.4, and containing 115 mM KCl, 10 mM $MgCl_2$, and 2 mM

EDTA, and subsequently pooled together. Thus, for each placenta a single homogenate was obtained. All homogenates were centrifuged at 9500×g for 10 min at 4°C. When the sediment was discarded, the total PK activity was determined in all supernatants. Afterwards, all supernatants were dialyzed against the buffer used before for 24 h at 4°C. PK activity was again determined in all fluids obtained after dialysis.

For the isolation of the PK isoenzymes, the fluids obtained after dialysis were pooled together within each group. The procedure of purification of the PK isoenzymes was carried out as described previously [9,18–22]. The resulting three fluids were subjected to fractional precipitation with ammonium sulfate. In each group, two precipitates were formed at the ammonium sulfate saturation values of 21–30% and 51–70%. In agreement with the method used, the precipitates corresponded to the L and M_2 isoenzymes, respectively. The precipitates were dissolved in a buffer consisting of 20 mM Tris-HCl, pH 7.4, containing 115 mM KCl, 10 mM $MgCl_2$, and 2 mM EDTA, and then they were dialyzed for 24 h at 4°C using the same buffer. For further purification of both isoenzymes, six samples were separated electrophoretically by the Davis's method [23], using a 7.5% polyacrylamide gel (in 0.005 M Tris-glycine buffer with the addition of 0.038 M glycine for polymerization). Electrophoresis was carried out for 2 h at 4°C in 0.01 M Tris-glycine buffer with 0.076 glycine, pH 8.4, at 180 V and 2 mA per sample. In the course of each electrophoretic separation, standard preparations of both isoenzymes were used (Sigma and Boehringer, Mannheim, Germany). After termination of electrophoresis, the gels were cut into 1.5-mm-thick strips. These were put into separate test-tubes, to which 0.2 ml of 75 mM Tris-HCl buffer, pH 7.4, containing 100 mM KCl, 25 mM $MgCl_2$, and 2 mM EDTA, was added. The extraction lasted 24 h at 4°C. In the resultant extracts, as during all the previous stages of purification, determinations were made of the PK activity and protein content. The extracted fluids (2 or 3 test tubes) with the most potent PK activity were pooled together, yielding preparations of purified isoenzymes.

PK activity was estimated by the spectrophotometric method developed by Bücher and Pfleiderer at 25°C [24]. The assay was carried out at 340 nm, the kinetics being kept at a pseudo-zero order. A 5 mM solution of 2-phosphoenolpyruvate (Calbiochem, La Jola CA, USA), dissolved in the 75 mM Tris-HCl buffer, pH 7.4, containing 100 mM KCl, 25 mM $MgCl_2$ and 2 mM EDTA, was used as a substrate. The reaction was started by adding 1.25 mM of ADP solution (Boehringer, Mannheim, Germany). The protein concentration was measured by the method of Lowry et al. [25].

Of the allosteric effectors affecting the PK activity, ATP (Boehringer, Mannheim Germany)and L-cysteine (Serva Feinbiochemical, Germany) were chosen. The effect of lead (+2) acetate and potassium fluoride on PK activity was also studied. In the preincubation mixture, the concentration of ATP and L-cysteine was 0.1 mM, while that of lead (+2) acetate was 100 μg/l and of potassium fluoride – 50 μg/l. The results were expressed in IU/g protein.

The results obtained were statistically analyzed using the t-Student's test.

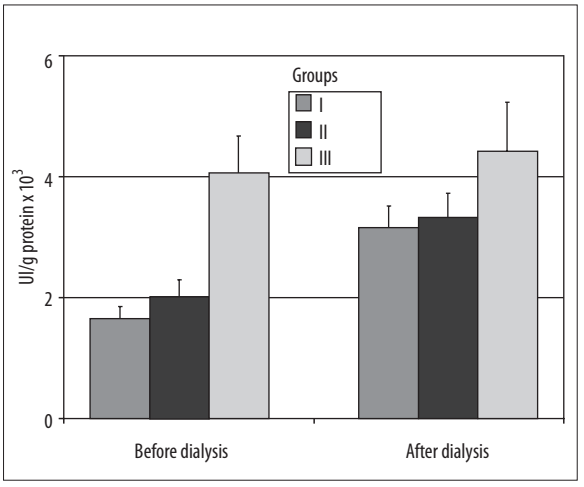

Figure 1. Total PK activity in the placentas of women inhabiting polluted areas (Group I – 6 placentas, Group II – 9 placentas) and those living in a pollution-free environment while pregnant (Group III – 8 placentas). The mean ±SD values in the supernatants obtained by centrifugation of the homogenates and in the fluids after dialysis of the supernatants are given.

RESULTS

Total PK activity in the placentas of the investigated women

In the supernatants of the placentas of women assigned to Groups I and II, total PK activity was lower than in the supernatants of the placentas of Group III women, regarded as the reference group (Figure 1). The mean value was the lowest in Group I (1.65±0.20×10³ IU/g protein), higher in Group II (2.02±0.28×10³-IU/g protein) and the highest in Group III (4.06±0.61×10³ IU/g protein). Total PK activity in Groups I and II amounted to 59% and 50% of the Group III value, respectively. The differences in the mean values of PK activity between Groups I and III as well as between Groups II and III were statistically significant ($p<0.001$).

Total PK activity in post-dialysis fluids obtained from supernatants of the placentas of women belonging to the three investigated groups was higher after dialysis than before (Figure 1). The lowest mean value (3.16±0.36×10³ IU/g protein) was found in Group I, higher (3.33±0.40×10³ IU/g protein) in Group II, and the highest (4.42±0.81×10³ IU/g protein) in Group III. However, the mean post-dialysis values of total PK activity calculated for the particular groups showed smaller differences than the pre-dialysis values. The increase in total PK activity in the post-dialysis fluids was considerably higher in Groups I and II than in Group III. The mean value of PK activity was twice as high in Group I, and almost twice as high in Group II than before dialysis. In contrast, in the reference Group III the increase in total PK activity was the lowest and did not exceed 10%. In spite of such a small increase, post-dialysis PK activity was still the highest in Group III. After dialysis the differences in the mean values of total PK activity between Groups I and III as well as between Groups II and III were statistically significant ($0.01<p<0.001$).

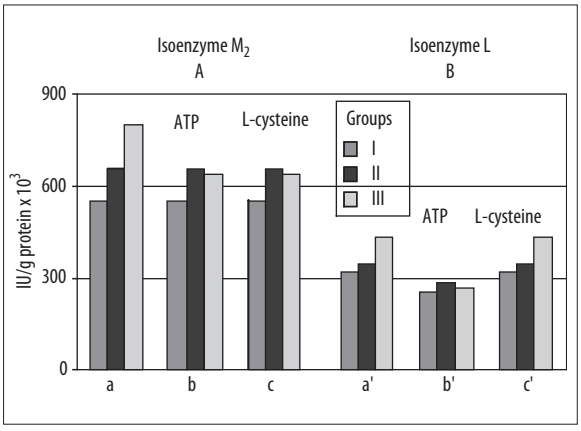

Figure 2. The decreases in the specific activities of M_2 and L isoenzymes by ATP and L-cysteine. Both isoenzymes were isolated from the placentas of pregnant women inhabiting polluted areas (Groups I and II) as well as from the placentas of women living in a pollution-free environment while pregnant (Group III). A: a) the initial values of M_2 isoenzyme-specific activity in Groups I, II, and III; b) values after addition of ATP, c) values after addition of L-cysteine; B: a') the initial values of L isoenzyme activity in Groups I, II, and III; b') values after addition of ATP, c') values after addition of L-cysteine.

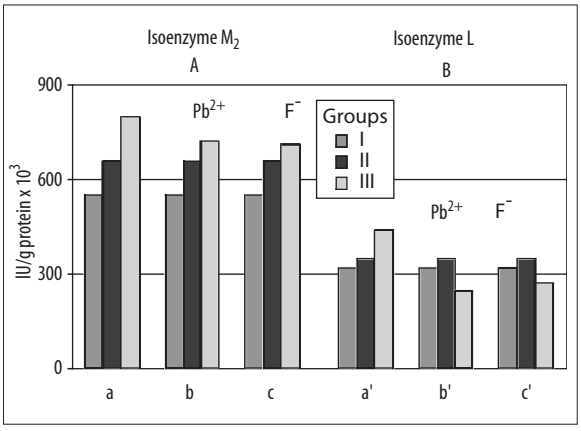

Figure 3. The inhibition of the specific activities of M_2 and L isoenzymes by lead (+2) and fluoride ions. Both isoenzymes were isolated from the placentas of pregnant women inhabiting polluted areas (Groups I and II) and from the placentas of women living in a pollution-free environment while pregnant (Group III). A: a) the values of M_2 isoenzyme activity in Groups I, II, and III; b) values after addition of lead (+2) ions; c) values after addition of fluoride ions. B: a') the initial values of L isoenzyme activity in Groups I, II, and III; b') values after addition of lead (+2) ions; c') values after addition of fluoride ions.

The PK isoenzymes in the three investigated groups

In each of the three fluids obtained after dialyzing the supernatants from the placentas of the women belonging to the three studied groups, two precipitates were obtained, one following the saturation of the solution with ammonium sulfate at the value of 21–30%, and one at 51–70%. In agreement with the procedure used, these corresponded to the L and M_2 isoenzymes. No intermediate fraction was observed to occur, which normally precipitate within the range of 31–50% of ammonium sulfate saturation and would correspond to the M_1 isoenzyme. The M_2 and L isoenzyme-containing sediments were subsequently purified electrophoretically. In total, each isoenzyme was purified approximately one hundred times. In the three investigated groups, two isoenzymes, namely M_2 and L, were always present.

The M_2 isoenzyme from the placentas of women belonging to particular groups showed different values of specific activity (Figure 2). The lowest specific activity (550×10^3 IU/g protein) was characteristic of the M_2 isoenzyme purified from the placentas from Group I women. A higher specific activity (660×10^3 IU/g protein) was noted in that from the placentas of Group II women, while the highest values (800×10^3 IU/g protein) was observed in that isolated from the placentas of Group III women. As in the case of the M_2 isoenzyme, the L isoenzyme obtained from the placentas of Group I showed the lowest specific activity (320×10^3 IU/g protein). That originating from Group II placentas demonstrated a slightly higher specific activity (350×10^3 IU/g protein), while the highest value (440×10^3 IU/g protein) was noted in that of Group III women. Using 2-phosphoenolopyruvate as a main substrate and the same method for determining the activity of both isoenzymes, the authors noted that in each group the M_2 isoenzyme showed a higher specific activity than the L isoenzyme.

The M_2 isoenzyme purified from the placentas of Group I and II women was not negatively affected by ATP. In contrast, ATP exerted a negative effect on the M_2 isoenzyme from the placentas of Group III women. Its specific activity was reduced by 20%. The M_2 isoenzyme originating from the placentas of Group I and II women was insensitive to L-cysteine. Only in the reference Group III did this amino acidic diminish the specific activity of the isoenzyme by 20%. On the other hand, L isoenzyme was negatively affected by ATP in all the three investigated groups (Figure 2). The activity of the L isoenzyme obtained from the placentas of Group I and II women was reduced by this mononucleotide by 20% and 17%, respectively. The highest decrease in this specific activity (40%) was observed in Group III. In none of the investigated groups was L isoenzyme affected by L-cysteine (Figure 2).

In the concentrations used, lead (+2) and fluoride ions did not inhibit the M_2 isoenzyme from the placentas of Group I and II women (Figure 3). Only in Group III did both ions inhibit the M_2 isoenzyme. The decrease in its specific activity triggered by lead (+2) and fluoride ions amounted to 12% and 11%, respectively. In turn, the L isoenzyme obtained from the placentas of Group I and II women was inhibited neither by lead (+2) nor by fluoride ions (Figure 3). Only in Group III did the ions result in inhibiting this isoenzyme. The decrease in the specific activity of the L isoenzyme was 44% and 40%, respectively.

Discussion

We have shown that in the placentas of women inhabiting a polluted environment (Groups I and II) total PK activity was significantly lower than in the placentas of women living in a pollution-free environment while pregnant (Group III). Diamant and Shafrir [26] demonstrated a decreased total

activity of PK in the placentas of rats fasted for 48 h as well as an increased activity of this enzyme in the placentas of diabetic rats compared with a control group. Thus it seems that in animals, under unfavorable conditions, total PK activity might be changed in the placenta. One may, therefore, surmise that a similar phenomenon occurs in the human placenta. In turn, total PK activity in the placentas of Group III women was comparable to the activity of this enzyme in mouse embryos (4.7×10^3 IU/g protein) previously determined in our laboratory, but was several times higher than in mouse and rat liver (0.43×10^3 and 0.90×10^3 IU/g protein, respectively) [10,15,16]. In addition, Hauguel-de Mouzon and Shafrir [27] demonstrated that total PK activity in the placentas of several animal species, as well as in man, was higher than in the liver. As it follows from this observation, the rate of the third stage of glycolysis in the placenta may be even higher than in the liver. This phenomenon possibly results from the necessity of producing lactate in the placenta.

Total PK activity in post-dialysis fluids obtained from the supernatants of the placentas of women belonging to the three investigated groups was found to be higher after dialysis than before. The increase in total PK activity was substantially higher in Groups I and II than in Group III. The placentas of women in Groups I and II probably contained a considerable amount of low-molecular-weight substances and ions, which are removable by dialysis. Binding in the allosteric or catalytic center of PK, these substances might limit its activity. Factors limiting total PK activity may include lead (+2) ions, which react with thiol groups of various proteins, including pyruvate kinase. Hence, the binding of the cysteine side chain with lead (+2) ions may restrict the activity of this enzyme. Ikeda and Noguchi [28] demonstrated that cysteine 423 was necessary for the allosteric properties of PK to occur. The supposition that lead (+2) ions may bind thiol groups in the allosteric center of PK is also confirmed by the present results. In comparison with values obtained in Groups I and II, the increase in total PK activity was small in the fluids after dialysis in Group III women. Thus the presence of these ions in the placentas of women living in a polluted environment may diminish total PK activity.

The passage of lead (+2) ions from the maternal blood to the placenta in women inhabiting polluted environments was confirmed by some researchers in several countries with diversified air pollution levels (29–33). Gumińska and Libik [34] observed that lead (+2) ion concentration was significantly higher in the placentas of women inhabiting polluted areas than in the placentas of women living in pollution-free areas. The study was carried out on the placentas of women living in the same areas as the subjects of the present investigation. Based on the results obtained in our laboratory and those presented by other authors, one might state that the composition of the polluted air, as well as the degree of air pollution, may affect the concentration of lead (+2) ions in the placenta. In turn, the total activity of PK is dependent on the placental concentrations of these ions.

Of the four known isoenzymes of PK, two, namely M_2 and L, were purified for the first time ever from the placentas of women belonging to the three investigated groups. Spellman and Fottrell [12] previously found only M_2 isoenzyme in the placentas of healthy women. This observation is in part contrary to the results of the present report. The difference most likely results from a different method of PK isoenzyme isolation. In the placentas of women living in polluted areas as well as in the controls, the specific activity of M_2 isoenzyme was approximately two times higher than that of L isoenzyme. Thus, M_2 is the main isoenzyme in the placenta, as well as in other fast-growing tissues [14–17]. The same two PK isoenzymes have also been demonstrated in the liver, but in the liver the activity of L isoenzyme is higher than that of M_2 isoenzyme [9,15,16]. It follows that there is an inverse relationship between both isoenzymes in these organs. This inverse ratio of the activity of these isoenzymes in the placenta and liver most likely ensures a specific regulation of the rate of the third glycolysis stage in each of these organs. In the placenta, the regulation of the specific activities of M_2 and L isoenzyme depends to a great measure on the substrate and allosteric effector concentrations. In turn, the hormonal effect is stronger in the liver, especially with respect to L isoenzyme.

The effect of ATP on M_2 isoenzyme obtained from the placentas of women inhabiting polluted areas was different from that on the same isoenzyme originating from placentas of women living in pollution-free regions. As previously demonstrated, M_2 isoenzyme isolated from different tissues, among them from the placenta, may occur in two forms, one composed of isoenzyme molecules with a dimeric structure, while the other consists of tetrameric molecules. In view of the value of the isoelectric point, the first form has been termed "alkaline" and the other "acidic", the latter showing a higher affinity to 2-phosphoenolopyruvate and being negatively affected by ATP [9–11]. The "alkaline" form has been assumed to predominate in the placenta [12]. The lower specific activity of the M_2 isoenzyme purified from the placentas of Group I and II women compared with the controls resulted from the fact that in these groups only the "alkaline" form of the isoenzyme was present. Most likely, when the conditions were unfavorable for the placentas, the contaminants hindered the binding of dimers to tetramers. Thus the "alkaline" form of the M_2 isoenzyme was unable to bind with ATP and, therefore, did not undergo the allosteric regulation evoked by this effector. On the other hand, the ATP-triggered reduction of the specific activity of the M_2 isoenzyme originating from Group III women by 20% leads to the assumption that, apart from the "alkaline", also the "acidic" form of this isoenzyme was present. Most likely, in the placentas of women inhabiting pollution-free areas, tetramers of the M_2 isoenzyme are more easily formed and they are subject to allosteric regulation by ATP.

Similar to the case of ATP, the effect exerted by L-cysteine on the M_2 isoenzyme purified from the placentas of women living in a polluted environment was different from that of the same isoenzyme originating from the placentas of women inhabiting pollution-free areas. As previously demonstrated in our laboratory, L-cysteine is a stereo-specific negative effector of the "alkaline" form of the M_2 isoenzyme from neoplastic tissues [16,35]. However, it was proven that the "alkaline" form of the M_2 isoenzyme obtained from the 7777 Morris hepatoma was not uniform and consisted of three variants, termed α, β and γ, whose movement rates in an electric field were variable. Only the slowest variant, γ, was sensitive to L-cysteine [22]. Since the M_2 isoenzyme puri-

fied from the placentas of Group I and II women did not bind with L-cysteine, this isoenzyme was probably a mixture of the α and β variants, or consisted purely of one of them. On the other hand, the 20% decrease in the specific activity of the M_2 isoenzyme purified from the placentas of Group III women caused by L-cysteine allows the assumption that apart from the α or β, the γ variant was also present.

Similarly as with M_2 isoenzyme, the same allosteric effectors were employed to evaluate the properties of L isoenzyme. The L isoenzyme is a tetramer that has allosteric properties, and ATP is one of its negative effectors. This isoenzyme also undergoes a covalent modification via phosphorylation or dephosphorylation occurring within each subunit at the serine residue close to the N-terminal of the molecule. The L dephospho-isoenzyme shows a higher affinity to 2-phosphoenolopyruvate and thus is more active than the phosphorylated form. Nevertheless, ATP binds more easily with the phospho- than with the L dephospho-isoenzyme [36,37]. The L isoenzyme purified from the placentas of the women of the three investigated groups was negatively affected by ATP, but the degree of such effect was variable. One may state that the placentas of Group I and II women contained more L dephospho-isoenzyme, whereas in the placentas of Group III women the L phospho-isoenzyme prevailed. Most likely in the placentas of women inhabiting areas with polluted air, when only the "alkaline" form of the M_2 isoenzyme was present, the production of a sufficient amount of pyruvate was made possible by the L dephospho-isoenzyme. In turn, in the placentas originating from women living in a pollution-free environment, in which both the "alkaline" and "acidic" forms of the M_2 isoenzyme were present, there was a greater amount of the L phospho-isoenzyme.

L isoenzyme purified from the placentas of the three investigated groups was not affected by L-cysteine. It was previously shown that the L isoenzyme from the liver was also insensitive to L-cysteine [16]. Thus the insensitivity of L isoenzyme to L-cysteine was similar in both organs. The authors decided there was no need to employ this amino acid while evaluating the properties of L isoenzyme.

It was previously demonstrated that lead (+2) and fluoride ions found in a polluted environment might penetrate the placenta from the maternal blood [29–34]. Therefore, the authors studied the effect of these ions on the M_2 and L isoenzymes purified from the placentas of the three investigated groups of women. The M_2 and L isoenzymes obtained from the placentas of Group I and II women were inhibited neither by lead (+2) nor by fluoride ions at the concentrations used, whereas these ions inhibited both isoenzymes from the placentas of Group III women. Most likely, pollutants, including lead (+2) and fluoride ions, in the placentas of Group I and II women bind with these isoenzymes, which may lead to some changes on the surfaces of their molecules. However, no decreased activity was observed following the administration of lead (+2) or fluoride ions to the M_2 and L isoenzymes originating from the placentas of Group I and II women. On the other hand, the addition of lead (+2) or fluoride ions to the M_2 and L isoenzymes obtained from the placentas of Group III women resulted in inhibition of their activity. In the case of M_2 isoenzyme, the activity was only slightly decreased, while the activity of L isoenzyme showed an almost 40% inhibition. Thus, the L isoenzyme was more sensitive to these ions used.

In summary, our results indicate that following the exposure of pregnant women to various environmental pollutants, the placenta shows a depressed total PK activity. A negative effect of pollutants has been confirmed by dialysis. The authors have demonstrated an increase in total PK activity in the supernatants of placentas of Group I and II women, as well as only a slight increase in the activity in the Group III controls. The decrease in placental PK activity leads to the formation of a smaller amount of pyruvate, what decelerates the rate of substrate-level phosphorylation in the cytoplasm. The reduction of pyruvate to lactate is also slower in consequence of a diminished activity of lactate dehydrogenase [6,7,38]. The lower than normal amount of lactate results in a slower regeneration of the NAD^+ dinucleotide from NADH, which hinders the course of glycolysis when the placental availability of oxygen is limited. Also, the amount of lactate that reaches the fetus is smaller. In addition, a lower than normal amount of pyruvate is transported to the mitochondria when PK activity is decreased. In turn, an insufficient amount of pyruvate in the mitochondria leads to a reduction in the rate of the Krebs cycle and the respiratory chain. This results in the formation of a smaller amount of ATP, also via oxidative phosphorylation. The real ATP concentration within the adenile pool in the cytoplasm is then lower. Studies on the total activity of PK, which is one of the key enzymes of glycolysis, may contribute to a better understanding of carbohydrate metabolism in the placenta. Based on the reports of Diamant [39], Page [40], Matsubara and Sato [41], Matsubara et al. [42], and Haugel-de Mouzon and Shafrir [27], it may be assumed that there is a close interrelation of metabolic pathways of the carbohydrate metabolism in this organ.

CONCLUSIONS

After dialysis of samples, a significant increase in total PK activity in the placentas of Groups I and II was observed, while Group III showed only a slightly elevated PK activity. Total PK activity in the placentas of women who had lived in a polluted environment while pregnant (Groups I and II) was lower than in the placentas of women inhabiting pollution-free areas (Group III). The authors were the first ever to demonstrate the presence of two PK isoenzymes, namely M_2 and L, in the placentas. Despite the lower activity of total PK, the presence of the two isoenzymes made it possible to produce a sufficient amount of pyruvate, which is major substrate of mitochondrial oxidative phosphorylation.

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